BIOLOGICAL CONTROL OF *DRECHSLERA TERES*: ABILITY OF ANTAGONISTS TO REDUCE CONIDIA FORMATION, COLEOPTILE INFECTION AND LEAF INFECTION IN BARLEY *(HORDEUM VULGARE)*

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ABSTRACT Preventive seed application of an Actinomycete, *Trichoderma viride*, *Myrothecium verrucaria* or *Trichoderma pseudokoningii* reduced seed borne *Drechslera teres* infection of barley coleoptiles by 90%, 87%, 79% and 76%, respectively. Moreover, leaf infection after artificial inoculation was decreased by 87%, 84%, 79%, 77% and 71% when the Thibaut cultivar had been previously treated at the three leaf stage with *Myrothecium verrucaria*, Actinomycete, *Trichoderma* sp. *Trichoderma viride* or *Trichoderma pseudokoningii* respectively. These antagonists also reduced conidia formation on straw colonized by *D. teres*.

RÉSUMÉ L'étude sur orge a confirmé l'efficacité des germes antagonistes (Actinomycete, *Trichoderma viride*, *Myrothecium verrucaria*, *Trichoderma* sp. et *Trichoderma pseudokoningii*) sur les trois séquences épidémiologiques majeures de l'helminthosporiose causée par *Drechslera teres*. L'inoculum séminicole (réduction du niveau d'attaque coléoptilaire par l'Actinomycète 90%, *Trichoderma viride* 87%, *Myrothecium verrucaria* 79% et *Trichoderma pseudokoningii* 76%). La contamination aérienne (diminution de l'importance et du nombre de lésions foliaires *Myrothecium verrucaria* 87%, Actinomycete 84%, *Trichoderma* sp. 79%, *Trichoderma viride* 77% et *Trichoderma pseudokoningii* 71%). Pour la conidiogénèse sur pailles, on note des actions antagonistes significatives de *Myrothecium verrucaria* et de l'Actinomycète appliqués sur les résidus de récolte.

KEY WORDS biological control, barley, antagonism, barley net blotch, *Hordeum vulgare*, *Drechslera teres*

INTRODUCTION

Drechslera teres (Sacc.) Shoem. (anamorph of *Pyrenophora teres* f. *teres* Drechs.) is the causal agent of barley net blotch. This cereal disease can cause considerable damage in a number of barley growing areas in various parts of the world (Smedegard-Petersen, 1974, Sutton & Steele, 1983, Martin, 1985). The yield reduction in barley plants infected with *D. teres* results mainly from decreased weight of grain, whereas the number of grains per plant is usually less affected (Smith et al., 1988).

This pathogen predominantly attacks the leaf and overwinters on infected debris and grain Inoculum destruction and barriers to prevent colonization of the plant by the pathogen are of primary importance for efficient control of the disease (Shipton et al., 1973)

Biological control, in its broadest sense, which includes rotations and other agricultural practices, has been used since prehistory (Campbell, 1990) The direct use of micro-organisms isolated from the soil to inoculate plants and reduce the disease was first reported in a series of papers around 1920-1930 (Campbell, 1990), but these effects remained largely a scientific curiosity until comprehensive books (Baker & Cook, 1974, Cook & Baker, 1983 and Mukerji & Garg, 1988) collected and analysed the available knowledge and stimulated further research, which resulted in many laboratory investigations but few effective field trials (Campbell, 1990)

Treatment of wheat and barley seeds with *Streptomyces griseoviridis* decreased damage caused by *Fusarium* spp and *Bipolaris sorokiniana* on both inoculated and uninoculated seeds (Tahvonen & Avikainen, 1990) Antagonists isolated from the resident microflora would seem to be good candidates for controlling foliar diseases, especially if a strategy of early introduction and establishment as residents is to be followed (Spurr, 1981).

Certain groups of bacteria, including fluorescent *Pseudomonas*, *Xanthomonas* and the *Erwinia* spp , are in relatively high densities on leaf surfaces and non pathogenic members of these groups have been recognized as potential biocontrol agents (Mukerji & Garg, 1988)

Apart from bacterization, inoculation of seeds with fast growing fungi can prevent seed decay and seedling blight Inoculation of corn seeds with *Chaetomium globosum* has resulted in field control of seedling blight caused by *Fusarium graminearum* Similarly oat seeds coated with *Chaetomium* spp. have some provided control of *Helminthosporium victoriae* (Purkayastha, 1989)

The aim of the present study was to assess the ability of some antagonists against *D. teres* on barley to suppress conidia formation and to reduce both coleoptile and leaf infection

MATERIAL AND METHODS

Antagonists

Isolates of *Trichoderma pseudokoningii*/N69, *Trichoderma viride*/NRG-1, *Trichoderma* sp./BRL 124, *Myrothecium verrucaria*/N76-1 and actinomycete/N51 were selected from 50 strains of fungi, actinomycetes and bacteria isolated from soil and straw obtained from the Department of Phytopathology, ENSAT, Toulouse University, France and tested *in vitro* for their ability to control net blotch caused by *D teres* (Mostafa et al , 1992) These fungal isolates were grown on PCA (potato 20 g, carrot 20 g and agar 20 g in 1000 ml distilled water) in Petri dishes for 15 days using agar disks from stock culture under laboratory conditions

Actinomycete/N51 was grown in a liquid culture medium (meat extract 2 g, peptone 2 g and NaCl 5 g in 1000 ml distilled water) for 8 days under laboratory conditions as shake cultures Aqueous suspensions of all the antagonists were prepared and adjusted to 1 x 10^6 propagules/ml with a haemocytometer

Pathogen

A 5 mm disk of mycelium of *D teres* (isolate R3) was taken from the edge of 8-day-old fungal colony on PCA in a Petri dish and cultured in 50 ml of 10% V8 liquid medium. After incubation at 23°C for 10 days, the mycelium was ground in water with a blender for 1 min to produce approximatively 1 x 10^4 mycelial fragments per milliliter. Gelatin (0 25%) and Triton X100 (one sticker drop per 100 ml) were added

Treatment of barley seeds

Seeds of barley cultivar Thibaut were surface sterilized using 95% ethanol (5 min) then 1% $HgCl_2$ (3 min) and carefully rinsed in sterilized distilled water (5 x 5 min) The seeds were dipped into the propagule suspensions of the antagonists and allowed to dry for 24 h After dipping the seeds into mycelium suspensions of *D teres* they were placed in test tubes containing cotton soaked in a nutrient solution ($NaNO_3$ 1 g, KNO_3 0 25 g, $MgSO_4$ 7 H_2O 0 25 g, KH_2PO_4 0 25 g and $FeCl_3$ 0 001 g, in distilled water 1000 ml), and the tubes were incubated at 18°C with 12h daylength When the 2nd leaf was almost fully expanded, the necrotic areas on the coleoptiles were examined A control set of seeds was inoculated with *D teres* alone and another set with sterilized distilled water Each treatment included ten replications (10 x 5 seeds)

Treatment of barley leaves

Seeds of barley cultivar Thibaut were sown in pots (10 seeds/pot, with 6 replicates per treatment), and when the 3rd leaf was almost fully expanded spore suspensions of the antagonists were sprayed over the upper surface of all the leaves Then after incubation under polyethylene bags for 24 h at 18°C, the leaves were inoculated with mycelial suspensions of *D teres* The pots were again covered with polyethylene bags, for 48 h, and then transferred to a glasshouse for disease development Throughout the experiments, the temperature was in the range 18 20°C, and the relative humidity was 85-95% with a 12 h daylength (light intensity = 60 Wm^{-2})

After 15 days, the incidence% (number of leaves infected per treatment) and severity (leaf area infected using a scale (Table 1) from 0 (no disease) to 9 (maximum disease) were evaluated and the coefficient of infection (CI) was calculated by multiplying the incidence by the severity (Loegering, 1959).

Treatment of barley straw

Samples of barley cultivar Thibaut stubble were collected from the fields and the straw naturally covered with conidiophores of *D teres* was cut into 8 cm lengths These were soaked in conidial suspensions of the antagonists (1 x 10^6 conidia/ml), and a control sample was soaked in sterilized distilled water Three replicated Petri dishes were set up with 10 fragments of straw per dish (100% RH, 12 h daylength, 18°C), and covered so as to promote conidia production After 4 and 6 days, conidiophores and conidia were counted microscopically after washing them with 25 ml sterilized distilled water

A statistical analysis was carried out on data using arc sin transformation (Snedecor & Cochran, 1971)

LEAF AREA INFECTED %	SCORE	SYMPTOM DESCRIPTION
0	0	no visually observable infection of the leaf
0 - 2.5	1	minute to small lesions : minute necrotic areas that may or may not be accompanied with chlorosis of surrounding tissues
2.5 - 5	2	
5 - 10	3	
10 - 20	4	small to medium sized lesions : necrotic areas medium in size and surrounded by chlorotic zones
20 - 30	5	
30 - 40	6	
40 - 50	7	
50 - 75	8	medium to large lesions : heavily infected leaves with large necrotic areas surrounded by large chlorotic areas ; blighted and dying leaves
75 - 100	9	

Table 1 Grading and description of net blotch infection symptoms (determined by visual observation, Barrault, 1989)

Tableau 1 - Classes et description de symptômes foliaires induits par l'helminthosporiose de l'orge, Barrault, 1989)

RESULTS

Seed treatment

Table 2 shows that the most severe symptoms (as defined by the extent of necrotic areas) on the coleoptile were observed on the seeds inoculated with *D teres* alone (97 8%) All the antagonists used reduced the *D teres* coleoptile infection significantly ($P < 0.05$) Seed applications of actinomycete/N51 and *Trichoderma viride*/NRG1 gave the best control the severity of the attack by *D teres* was reduced to 9.8% and 13%, respectively.

The mycelial fragments sampled at various locations on the necrotic coleoptiles and cultured on V8 agar belonged to express the pathogen *D teres* in every case

Leaf treatment

Application of antagonists 1 day before inoculation of the leaves by a mycelial suspension of *D teres* significantly reduced the area of the net blotch lesions which subsequently developed on the leaves Table 3 shows that the coefficient of infection (CI) in the control plants (untreated with the antagonists) was much higher than in the plants treated with the antagonists The most significant reduction of leaf symptoms was obtained with *Myrothecium verrucaria*/N76 1 and actinomycete/N51 which reduced the necrotic leaf area by 95%

Parameter / Treatment	Mean coleoptile length (cm)	Mean length of necrotic areas per coleoptile per tube (cm)	Attack %
actinomycete/N51	5.1	0.5	9.8 c
Trichoderma viride/NRG1	4.6	0.6	13.0 cd
Myrothecium verrucaria N76-1	4.7	0.9	19.6 bc
Trichoderma pseudo-koningii/N 69	4.6	1.1	23.9 bc
Trichoderma sp/BRL 124	4.6	1.5	32.6 b
Control with *D.teres* alone	4.6	4.5	97.8a
Control with sterilized distilled water alone	4.6		0

Table 2 - Effect of seed treatment on reduction of coleoptile infection by *D teres*
The values (averages of 10 replicates with 5 plants/tube) which are followed by the same letter are not significantly different at the 5% level using the test of Newman-Keuls

Tableau 2 Efficacité des germes antagonistes sur la réduction du niveau d'attaque coléoptilaire par *D. teres*

Straw treatment

The ability of the antagonists to suppress conidia formation in *D teres* was tested in the laboratory using infected straw treated with antagonists The search for the more effective antagonists (Table 4, showed that *Myrothecium verrucaria*/N76 1, *Trichoderma viride*/NRG 1 and actinomycete/N51 could significantly ($P < 0.05$) reduce the number of conidia as compared with the untreated control.

Approximatively 79%, 75% and 70% of the conidia of *D. teres* were suppressed in straw after 4 days and 76%, 81% and 85% after 6 days in the presence of *Myrothecium verrucaria*/N76 1 *Trichoderma viride*/NRG-1 and Actinomycete/N51, respectively

DISCUSSION

The *in vivo* experiments presented above have shown that three saprophytic microorganisms (actinomycete/N51, *Trichoderma viride*/NRG-1 and *Trichoderma pseudokoningii*/N69) are efficient at three different stages in the life cycle of *D teres*

Treatment	Parameter: % Number of leaves infected per treatment	Leaf area infected per treatment	Coefficient of infection	Disease reduction %
actinomycete/N51	8	2.0	16	95.3 a
Myrothecium verrucaria N76-1	10	1.6	16	95.3 a
Trichoderma viride/NRG1	18	2.7	48.6	85.7 ab
Trichoderma sp/BRL 124	20	2.9	58	82.9 ab
Trichoderma pseudo-koningii/N69	13	3.7	48.1	85.9 ab
Control with *D.teres* alone	50	6.8	340.0	0 c

Table 3 - Effect of antagonists on reduction of leaf lesion by *D. teres*
Leaf areas were determined visually and scored on a 0-9 scale (Table 1)
Disease reduction = percent reduction in coefficient of infection with respect to control
The values (means of 3 replicates with 10 plants/pot) which are followed by the same letter are not significantly different at the 5% level using the test of Newman-Keuls

Tableau 3 - Efficacité des germes antagonistes sur la diminution de l'importance de lésion foliaires de *D teres*

These antagonists markedly reduced the production of conidial inoculum on the straw They could also decrease significantly the intensity of the coleoptile attack by the seed-borne inoculum Downes (1977) had already shown that the application of a bacterium, *Erwinia herbicola*, on oat seeds prevented the appearance of coleoptile necroses induced by *Pyrenophora avenae* More recently, Vannaci & Pecchia (1986) reported that *Drechslera sorokiniana* on barley seeds could be partially inhibited by *Trichoderma harzianum* and *Chaetomium globosum*, whereas Al-Hasmini & Perry (1986) observed a reduction of the coleoptile attack by *Gerlachia nivalis* in barley after the application of a spore suspension of *Trichoderma viride*.

The antagonists selected also decreased the intensity of foliar symptoms (through a reduction of the coefficient of infection) Scharen & Bryan (1981) had shown the efficiency of a preventive foliar treatment with a bacterial suspension (*Bacillus licheniformis*, against *Drechslera teres* on barley Slessman & Leben (1976) had also observed that a bacterium (AN77) could reduce dramatically the foliar symptoms caused by *Helminthosporium maydis* on maize under controlled conditions but not in the field

Time after treatment	Sporulation after 4 days		Sporulation after 6 days	
Parameter / Treatment	Conidia per 100 conidiophores	% reduction	Conidia per 100 conidiophores	% reduction
Control	130 a		342 a	
M. verrucaria/N76-1	27.1 c	79	69.7 d	76.6
Trichoderma viride/NRG1	33.1 c	75	64.3 d	81.2
actinomycete/N	39.9 bc	70	49.6 d	85.5
Trichoderma sp/BRL 124	39.5 bc	70	88.0 c	74.3
Trichoderma pseudokoningii/N69	58.5 b	55	183.0 b	46

Table 4 - Reduction of *D teres* conidia formation on the straw by antagonists
Mean of ten replicates each replication consisted of 10 fragments of straw for which 100 conidiophores were scored
Reduction (%) in number of conidia with respect to control
The percentages followed by the same letter are not significantly different at the 5% level using the test of Newman-Keuls

Tableau 4 Efficacité des germes antagonistes sur la conidiogénèse de *D teres* sur les pailles

Among the three antagonists selected, actinomycete/N51 displayed the broadest spectrum of action since its efficiency in the three epidemiological sequences investigated was good in every case An increase in efficiency might be obtained through the association of two or three different antagonists as a result of the synergistic microorganisms (Mostafa, 1982).

The antagonistic activity of these microorganisms in other sequences of the disease, particularly survival, still need to be tested The necrotrophic character of the pathogen and the importance of the saprophytic phase require the control of the multiple survival forms which generate the primary inoculum The survival forms on straw are mostly responsible for the development of the disease on farm plots since the aerial exogenous inoculum, as a result of its dispersion over short distances only, can be considered as epidemiologically non significant (Pieming, 1968, Barrault, 1989) With in this context, the efficiency of the antagonists selected might be tested on the following survival forms *i* the resting mycelium (inhibition of the asexual morphogenesis, i e conidiophores and/or conidia), *ii* the sclerotia (inhibition of the myceliogenic acti

vity), and *iii* the perithecia (inhibition of the perithecium ascus and ascospores morphogenesis)

As to the action of the antagonists on the teleomorph, Pfender (1988) showed that a basidiomycete (*Limonomyces roseipellis*), isolated from a microbial community on straw originating from minimum tillage plots (where the disease was declining), inhibited the formation of perithecia of *Pyrenophora tritici repentis* on wheat

The biological control of the pathogen in all its survival forms should be part of an integrated control program aimed at reducing the inoculum pressure on the plot

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